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FACSIMILE TRANSMITTAL SHEET

TO: DR. SUMESH KAUSHAL	FROM: JUDY JARECKI-BLACK, PH.D., J.D.
COMPANY: USPTO	DATE: 18 DEC 2000
FAX NUMBER: 703-308-8724	TOTAL NO. OF PAGES INCLUDING COVER: 13
PHONE NUMBER: 703-305-6838	SENDER'S FAX NUMBER: 706-227-2180
RE: AGENDA FOR TELEPHONE INTERVIEW ON 19 DEC 2000 AT 3:00 PM	SENDER'S PHONE NUMBER: 706-227-1170 X 232 ← 703-305-4051 ↓

☐ URGENT ☐ FOR REVIEW ☐ PLEASE COMMENT ☐ PLEASE REPLY ☐ PLEASE RECYCLE

NOTES/COMMENTS:

The proposed Agenda for the telephone conference scheduled for 19 Dec 2000 (3 PM) is as follows:

Discuss independent claims 19, 27 and 35 with regard to the enablement provided by Applicants' Specification and Declaration under 37 CFR 1.132.

Attached for the Examiner's consideration is

- Executed Declaration under 37 CFR 1.132 by Dr. Jeff Rapp
- A *Curriculum vitae* for Dr. Jeff Rapp
- A copy of the Associate Power of Attorney for Dr. Judy Jarecki-Black filed by facsimile with the PTO on 20 November 2000 ✓

Please do not hesitate to call me if you have any questions. Otherwise, I will call you at 3PM on Tuesday 19 Dec 2000. Thank you for your assistance.

PATENTS
Attorney Docket No. 24011-0002

CERTIFICATE OF MAILING

I hereby certify that this paper is being deposited with the U.S. Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, DC 20231, on _____.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Ivarie et al : :

Application No: 09/173,864 : Group Art Unit: 1633

Filed: October 16, 1998 : Examiner: Sumesh Kaushal

Title: NOVEL TRANSGENIC BIRDS AND THEIR EGGS

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

DECLARATION UNDER 37 CFR 1.132

I, Jeffrey Rapp, Ph.D., hereby declare as follows:

1. I am a staff scientist employed by AviGenics, inc., of Athens, GA., licensee of the subject application, since 11 April 1996. I consider myself to be skilled in the art of avian transgenesis. My *curriculum vitae* is appended hereto.

2. The following reports experiments conducted by me personally, or by those working under my supervision, to produce a transgenic chicken expressing an exogenous protein, following the procedures set forth in the subject application of Ivarie, *et al.*

Declaration
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3. These experiments were designed to demonstrate the expression of human interferon in a chicken, as suggested at page 32, line 6 of the specification.

4. Following the teachings of Example 1 (Vector Construction) of the specification, an pNLB-CMV-IFN vector was created, substituting an IFN encoding sequence for the BL encoding sequence of the Example.

The DNA sequence for human interferon α-2b based on hen oviduct optimized codon usage was created using the BACKTRANSLATE program of the Wisconsin Package, version 9.1 (Genetics Computer Group. Inc., Madison, WI) with a codon usage table compiled from the chicken (*Gallus gallus*) ovalbumin, lysozyme, ovomucoid, and ovotransferrin proteins. The template and primer oligonucleotides listed in Table 1 were amplified by PCR with *Pfu* polymerase (Stratagene, La Jolla, CA) using 20 cycles of 94°C for 1 min., 50°C for 30 sec., and 72°C for 1 min. and 10 sec. PCR products were purified from a 12% polyacrylamide-TBE gel by the "crush and soak" method (Maniatis *et al.* 1982), then combined as templates in an amplification reaction using only IFN-1 and IFN-8 as primers. The resulting PCR product was digested with *Hind* III and *Xba* I and gel purified from a 2% agarose-TAE gel, then ligated into *Hind* III and *Xba* I digested, alkaline phosphatase-treated pBluescript KS (Stratagene), resulting in the plasmid pBluKSP-IFNMagMax. Both strands were sequenced by cycle sequencing on an ABI PRISM 377 DNA Sequencer (Perkin-Elmer, Foster City, CA) using universal T7 or T3 primers. Mutations in pBluKSP-IFN derived from the original oligonucleotide templates were corrected by site-directed mutagenesis with the Transformer Site-Directed Mutagenesis Kit (Clontech, Palo Alto, CA). The IFN coding sequence was then removed from the corrected pBluKSP-IFN with *Hind* III and *Xba* 1, purified from a 0.8% agarose-TAE Gel,

and ligated to *Hind* III and *Xba* I digested, alkaline phosphatase-treated pCMV-BetaLa-3B-dH. The resulting plasmid was pCMV-IFN which contained IFN coding sequence controlled by the cytomegalovirus immediate early promoter/enhancer and SV40 polyA site. To clone the IFN coding sequence controlled by the CMV promoter/enhancer into the NLB retroviral plasmid, pCMV-IFN was first digested with *Cla*I and *Xba*I, then both ends were filled in with Klenow fragment of DNA polymerase (New England BioLabs, Beverly, MA). pNLB-adapter was digested with *Nde*I and *Kpn*I, and both ends were made blunt by T4 DNA polymerase (New England BioLabs). Appropriate DNA fragments were purified on a 0.8% agarose-TAE gel, then ligated and transformed into DH5α cells. The resulting plasmid was pNLB-adapter-CMV-IFN. This plasmid was then digested with *Mlu*I and partially digested with *Blp*I and the appropriate fragment was gel purified. pNLB-CMV-EGFP was digested with *Mlu*I and *Blp*I, then alkaline-phosphatase treated and gel purified. The *Mlu*I/*Blp*I partial fragment of pNLB-adapter-CMV-IFN was ligated to the large fragment derived from the *Mlu*I/*Blp*I digest of pNLB-CMV-EGFP, creating pNLB-CMV-IFN.

5. Following the procedures of Example 2 (Production of Transduction Particles), transduction particles of pNLB-CMV-IFN were produced.

Senta packaging cells (Cosset *et al.*, 1990) were plated at a density of 3 x 10^5 cells/35mm tissue culture dish in F-10 medium (Life Technologies) supplemented with 50% calf serum (Atlanta Biologicals), 1% chicken serum (Life Technologies), 50 μg/ml hygromycin (Sigma), and 50 μg/ml phleomycin (CAYLA, Toulouse, France). These cells were transfected 24h after plating with 2 μg of CsCl-purified pNLB-CMV-IFN DNA and 5 μl of Lipofectin liposomes (Life Technologies) in a final volume of 800 μl Optimem (Life Technologies). The next day, medium from transfected Sentas was recovered and filtered

through a 0.45 micron filter. This medium was then used to transduce Isolde cells. 0.5 ml of the filtered medium recovered from Senta cells was added to 6 ml of F-10 (Life Technologies) supplemented as described above, in addition to polybrene (Sigma) at a final concentration of 4 μg/ml. This mixture was added to 10^6 Isolde packaging cells (Cosset *et al.*, 1990) plated on a 100mm dish the previous day, then replaced with fresh medium (as described for Senta growth) 4h later. The next day, the medium was replaced with fresh medium which also contained 200 μg/ml neomycin (G418, Sigma). Every other day, the medium was replaced with fresh F- 10 medium containing the supplements described above for Senta growth, and also 200 μg/ml neomycin. Seven to ten days later, single colonies were visible by eye, and these were picked and placed into 24 well dishes. When the 24 well dishes became confluent, medium was harvested and titered to determine the cell lines with the highest production of retrovirus. Harvested medium was also tested for the presence of IFN by IFN ELISA.

Isolde cell line producing the highest titer of IFN-encoding transducing particles was scaled up to six T-75 tissue culture flasks. When flasks were confluent, cells were washed with F-10 medium (unsupplemented) and transducing particles were then harvested for l6h in 14 ml/flask of F-10 containing 1% calf serum (Atlanta Biologicals) and 0.2% chicken serum (Life Technologies). Medium was harvested, filtered through a 0.45 micron syringe filter, then centrifuged at 195,000xg in a Beckman 60Ti rotor for 35 min. Liquid was removed except for 1 ml, and this was incubated with the pellet at 37°C with gentle shaking for one hour. Aliquots were frozen at –70°C. Transducing particles were then titered on Senta cells to determine concentrations used to inject embryos.

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6. Following the procedures of Example 3 (Production of Transgenic Chickens), chimeric birds were produced.

Approximately 300 White Leghorn (strain Line 0) eggs were windowed according to the Speksnijder procedure (U.S. Patent No. 5,897,998), then injected with ~7 x 10^4 transducing particles per egg. Eggs hatched 21 days after injection, and human IFN levels were measured by IFN ELISA from serum samples collected from chicks one week after hatch.

7. Following the procedures of Example 10 (Production of Fully Transgenic G1 Chickens), males were selected for breeding.

To screen for G_0 roosters which contained the IFN transgene in their sperm, DNA was extracted from rooster sperm samples by Chelex-100 extraction (Walsh *et al.*, 1991). DNA samples were then subjected to Taqman™ analysis on a 7700 Sequence Detector (Perkin Elmer) using the "neo for-l" (5'-TGGATTGCACGCAGGTTCT-3') and "neo rev-1" (5'-GTGCCCAGTCATAGCCGAAT-3') primers and FAM labeled NEO-PROBE1 (5'-CCTCTCCACCCAAGCGGCCG-3') to detect the transgene. Three G_0 roosters with the highest levels of the transgene in their sperm samples were bred to nontransgenic SPAFAS (White Leghorn) hens by artificial insemination.

Blood DNA samples were screened for the presence of the transgene by Taqman™ analysis as described above. Out of 1,597 offspring, one rooster was found to be transgenic (a.k.a. "Alphie"). Alphie's serum was tested for the presence of hIFN by hIFN ELISA, and hIFN was present at 200 nanograms/ml.

Alphie's sperm was used for artificial insemination of nontransgenic SPAFAS (White Leghorn) hens. To date 106 out of 202 (~52%) offspring contain the transgene as

detected by Taqman ™ analysis. These breeding results follow a Mendelian inheritance pattern and indicate that Alphie is transgenic.

This declaration is made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. §1001) and may jeopardize the validity of the application or any patent issuing thereon. All statements made of my own knowledge are true and all statements made on information and belief are believed to be true.

Jeffrey C. Rapp

Jeffrey Rapp, Ph.D.